

**A New Classification for the Leucosphyrus Group
of *Anopheles (Cellia)*¹**

E.L. Peyton^{2,3}

ABSTRACT. The Leucosphyrus Group of the genus *Anopheles*, subgenus *Cellia*, is divided into three subordinate groups based upon an analysis of relative lengths of the female proboscis, maxillary palpus and forefemur, and the relationships among them. The Elegans, Leucosphyrus and Riparis Subgroups are proposed and currently recognized taxa are assigned to each. Assignment of species to the Dirus and Leucosphyrus Species Complexes is also indicated. The relationships of the subgroups are figured, and a listing of currently recognized taxa belonging to the Leucosphyrus Group is tabulated.

INTRODUCTION

The Leucosphyrus Group of the genus *Anopheles* is of considerable medical and public health importance in Southeast Asia. At least three members of the group are known to be highly efficient vectors of human malaria parasites, i.e., *balabacensis* Baisas, *dirus* Peyton and Harrison and *leucosphyrus* Doenitz. Other members are suspected vectors and several are also known to transmit simian malaria parasites. A few works recording the historical importance of the Leucosphyrus Group species on malaria transmission include Clark and Choudhury (1941), McArthur (1947, 1951), Kuitert and Hitchcock (1948), Macan (1948), Colless (1950, 1956), Scanlon and Sandhinand (1965), Reid (1968) and Rosenberg and Maheswary (1982). Only in recent years has the public health significance of the group been fully realized, with *dirus* now viewed by most investigators as the most important vector on mainland Southeast Asia. As a result, there has been a considerable increase in interest and available resources for studying this group during the past 15 years. These studies now include all aspects of systematics, biology (behavioral and molecular) and epidemiology.

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The most recent taxonomic revision of the group was published more than 30 years ago (Colless 1956). In this publication and additional notes (Colless 1957), he recognized 6 species, 4 subspecies and 3 forms of doubtful status. Currently, 20 species and 2 geographic forms of uncertain status are recognized (Table 1). Historically, the taxonomy of the group has been very confused and complex, primarily because it is a fairly homogeneous group with members showing various degrees of morphological and biological similarity, as well as considerable morphological variation within each life stage. It was only after the discovery and subsequent description of *dirus* by Peyton and Harrison (1979) that a new insight into the taxonomic order of these taxa began to evolve. *Anopheles dirus* was recognized as a new species for what was previously known as *balabacensis* on the Southeast Asian mainland, at least for much of Thailand and neighboring countries to the east. It was then apparent that several different mainland populations of so-called *balabacensis* probably represented a complex of several species closely related to *dirus* and that true *balabacensis* probably did not occur on the mainland. This assumption has now been amply confirmed. Since the description of *dirus*, another new species has been described (Peyton and Ramalingam 1988), others have been redescribed in various life stages (Mendis et al. 1983(1984) and Hii et al. 1988) one has been removed from synonymy (Peyton and Harrison 1980) and two subspecies have been elevated to specific status (Hii et al. 1988). The Dirus Complex with seven assigned species (Table 1) was first characterized by Peyton and Ramalingam (1988). This group of species was previously regarded by various authors as belonging to the Balabacensis Complex. *Anopheles balabacensis*, however, is more closely aligned with *leucosphyrus* and is here, for the first time, assigned to the newly formed Leucosphyrus Complex and the Balabacensis Complex is no longer recognized.

The description of *dirus* in 1979 prompted numerous cytogenetic, cross-mating and biochemical studies on this species and closely related taxa in Thailand. Many of these studies were done in collaboration with this author. These studies primarily involved investigators at Mahidol University and the Armed Forces Research Institute for Medical Sciences (AFRIMS) in Bangkok. Voucher specimens have always been provided for this study, and are deposited in the Smithsonian Institution National Mosquito Collection which is curated by the Walter Reed Biosystematics Unit (WRBU). Several publications have resulted from these studies which include Baimai et al. (1980, 1981, 1984, 1988a, 1988b, 1988c), Hii (1982)⁴, Kanda et al. (1981), Hii (1985).

Almost all of the studies and resulting publications since Colless (1956) have dealt with the elucidation of individual species rather than analyzing the supraspecific categories of the Leucosphyrus Group. Most attention has centered on species of the Dirus Complex and many species of other groups have not been adequately addressed. This paper provides a new classification of the Leucosphyrus Group for the use of investigators in Southeast Asia. The systematic arrangement presented in Table 1 and Fig. 1 is based upon an analysis of several thousand specimens of all life stages from the entire range of the Leucosphyrus Group and also includes the observations of Colless (1956). I believe this arrangement is sound and I do not anticipate making any significant changes.

⁴Hii, J.L.K. 1982. Laboratory studies of three members of the *Anopheles balabacensis* complex (Diptera: Culicidae). Ph.D. Thesis, University of London. 252 pp.

CLASSIFICATION

Informal infrasubgeneric groupings have been used for many years in mosquito systematics, probably more so than in any other group of insects. Although these informal groups have no standing in zoological nomenclature and the *International Code of Zoological Nomenclature* (1985) does not regulate such categories, the practice has proven to be very useful and often has had considerable practical application. I believe such is the case with the groups proposed here. One of the great advantages of any practical utilitarian system of classification, whether proposed as formal or informal, is its usefulness in improving communication among those involved in the study of such groups. The informal categories now widely used by mosquito systematists include Sections, Series, Groups, Subgroups and Complexes. Some of these date to Christophers (1924) and Edwards (1932); they were extensively used with some modification in Knight and Marks (1952) for the subgenus *Finlaya* of *Aedes* and were standardized for the genus *Anopheles*, subgenus *Anopheles* by Reid and Knight (1961). Belkin (1962) and his associates followed this system which has been adopted subsequently by many systematists. Since all of the groups proposed here are informal and are not regulated by the *Code*, I choose to spell the group names in Roman type. Hence the expression Leucosphyrus Subgroup or Dirus Complex is treated as the vernacular name of a group or complex, respectively, even though the name of a species has been used in the combination. The intended meaning is totally unambiguous and eliminates the need for qualifiers for each instance of usage, as in the case of Colless (1956, 1957), Reid (1968) and others who enclosed the species name in quotes to denote reference to a group. The above practice is a useful convention which allows considerable flexibility while avoiding conflict with the more rigid application of the *Code*. This use does not preclude the possibility of elevating any given group to formal status if evidence supports the need to do so. I concede that this practice is not universally accepted, especially among other dipterists.

The Leucosphyrus Group is an easily recognized monophyletic aggregate of 22 taxa belonging to the Neomyzomyia Series of Christophers (1924). The Neomyzomyia Series is represented in the Afrotropical, Australasian and Oriental regions. The Series is generally characterized by the following morphological characters: adult female with a single row of rather large cibarial teeth not differentiated into rods and cones, maxillary palpus with four or more pale-scaled bands, wings sometimes with many small dark marks, usually with four or more on vein Cu-A, legs usually speckled, end of abdomen sometimes scaled; pupa with seta 1-P seldom long and hooked; larva with long pleural setae 9-12-P, M, T usually simple (Reid 1968).

The Leucosphyrus Group occurs only in the Oriental Region (Indomalayan and Oriental faunal regions of Belkin 1962). The group includes all taxa belonging to the Neomyzomyia Series which possess, in the adult stage, a very broad conspicuous white-scaled band covering the apex of the hindtibia and the base of hindtarsomere 1, legs speckled, wings with many discrete pale- and dark-scaled spots on all veins and with four or more dark spots present on vein Cu-A and terminal abdominal segments always with some scales.

Reid (1949) and Colless (1956) noted that in females of some species of the *Leucosphyrus* Group the maxillary palpus was noticeably shorter than the proboscis, while in other species the palpus and proboscis were approximately the same length. These authors also noted a similar correlation between the length of proboscis and the length of the forefemur. They used one or both of these lengths in combination in keys to separate various species. Colless compared the relative lengths of the female proboscis, palpus and forefemur as presented here. He presented (his Figure 13) an arrangement of species in decending order of proboscis/forefemur ratio. He also (his Figure 14) plotted the mean proboscis length of each taxon against mean forefemur length on a logarithmic scale. While he recognized groupings apparent in these measurements, stating "there appear to be three groups: (a) *A. pujutensis*, *A. hackeri*, the 'Celebes form', and *A. elegans*; (b) the *A. leucosphyrus* subspecies, with the exception of the 'Negros' and 'Luzon forms'; (c) these two latter forms (the 'Luzon form' is rather intermediate), the *A. riparis* subspecies and *A. cristatus*," he did not suggest names for them. He did, however, suggest that "using this grouping, a tentative phylogenetic scheme may be derived, using no mechanism of speciation other than that by geographic isolation, with subsequent divergence due to altered selection pressures and random 'drift'." Colless (1956) stated that "the position of the 'Negros form' is anomalous, but it has been plotted from a single poor specimen and may be inaccurate." I cannot add to this since I saw no additional specimens, however, it appears that this position is valid.

The groupings presented in Table 1 and Fig. 1 represent a confirmation and refinement of the groupings noted by Colless (1956). The recent addition of material of a new species of the *Elegans* Subgroup from Sumatra and many additional new members of the *Leucosphyrus* Subgroup were convincing evidence that the groupings noted by Colless (1956) were valid, useful divisions that would readily accommodate all known taxa. It is likely that any newly discovered taxa will be easily assigned to its proper group.

Some of the measurements and ratios presented in Fig. 1 differ slightly from those presented by Colless (1956), but this is only natural since many of these measurements were taken from material acquired after Colless completed his study, and in many cases material came from areas he did not cover. Any given sample will always vary slightly from a comparable sample but will usually fall within the parameters of the group or species, except for possibly an occasional anomalous specimen. While relative lengths and size are known to be strongly influenced by nutrition and other environmental factors during the development of the immature stages and, therefore, often vary greatly between individual specimens, the variation of individual morphological structures generally remain proportional to each other. Consequently, ratios and indexes of lengths and widths have been used widely and most effectively in systematics studies. In the case of the range for the *Riparis* Subgroup presented in Fig. 1, the ratios of proboscis/forefemur for samples from some of the same areas as those of Colless were 0.85-0.99. Colless showed a range of 0.88-1.00 ($\bar{x}=0.93$) for *macarthuri* from Borneo (18 specimens) and 0.91-1.03 ($\bar{x}=0.94$) for *macarthuri* from peninsular Malaysia (9 specimens). My sample of *macarthuri* had an overall range of 0.90-0.99 ($\bar{x}=0.95$) for Borneo (4 specimens), Peninsular Malaysia (18 specimens) and southern Thailand (18 specimens). On the other hand, Colless had only

one specimen of "Celebes form" (= *sulawesi*) with proboscis/forefemur ratio 1.26 and palpus/proboscis ratio 0.78 whereas my sample of 14 specimens showed means of 1.25 and 0.78, respectively, clearly demonstrating the rather uniform nature of the characters.

Figure 1 presents the basis for the groupings in Table 1. Figure 1 is self-explanatory. The method used for measuring the proboscis and maxillary palpus is that defined by Peyton and Ramalingam (1988). Hopefully this classification will help others to better understand the *Leucosphyrus* Group and will lead to a more uniform and logical use of terms for the subgroups and complexes within the *Leucosphyrus* Group. A detailed treatment of the morphology and taxonomy of the group is in progress, with species descriptions, biological and zoogeographic considerations which will reinforce this scheme of classification.

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REFERENCES CITED

- Baimai, V., R.E. Harbach and U. Kijchalao. 1988a. Cytogenetic evidence for a fifth species within the taxon *Anopheles dirus* in Thailand. *J. Am. Mosq. Control Assoc.* 4: 333-338.
- Baimai, V., R.E. Harbach and S. Sukowati. 1988b. Cytogenetic evidence for two species within the current concept of the malaria vector *Anopheles leucosphyrus* in Southeast Asia. *J. Am. Mosq. Control Assoc.* 4: 44-50.
- Baimai, V., B.A. Harrison and V. Nakavachara. 1980. The salivary gland chromosome of *Anopheles (Cellia) dirus* (Diptera: Culicidae) of the Southeast Asian *Leucosphyrus* group. *Proc. Entomol. Soc. Wash.* 82: 319-328.
- Baimai, V., B.A. Harrison and L. Somchit. 1981. Karyotype differentiation of three anopheline taxa in the Balabacensis complex of Southeast Asia (Diptera: Culicidae). *Genetica* 57: 81-86.
- Baimai, V., A. Poopittayasataporn and U. Kijchalao. 1988c. Cytological differences and chromosomal rearrangements in four members of the *Anopheles dirus* complex (Diptera: Culicidae). *Genome*, 30: 372-379.

- Baimai, V., C.A. Green, R.E. Andre, B.A. Harrison and E.L. Peyton. 1984. Cytogenetic studies of some species complexes of *Anopheles* in Thailand and Southeast Asia. *Southeast Asian J. Trop. Med. Public Health* 15: 536-546.
- Belkin, J.N. 1962. The mosquitoes of the South Pacific (Diptera: Culicidae). Univ. Calif. Press, Berkeley and Los Angeles. 2 vols. 608 and 412 pp.
- Christophers, S.R. 1924. Provisional list and reference catalogue of the Anophelini. *Indian Med. Res. Mem.* 3: 1-105.
- Clark, R.H.P. and L.M.P. Choudhury. 1941. Observations on *A. leucosphyrus* in the Digboi area, Upper Assam. *J. Malaria Inst. India* 4: 103-109.
- Colless, D.H. 1950. The identity of the malaria vector, *A. leucosphyrus*. *Indian J. Malariol.* 4: 377-383.
- Colless, D.H. 1956. The *Anopheles leucosphyrus* group. *Tran. R. Entomol. Soc. Lond.* 108: 37-116.
- Colless, D.H. 1957. Further notes on the systematics of the *Anopheles leucosphyrus* group (Diptera: Culicidae). *Proc. R. Entomol. Soc. Lond. Ser. B.* 26: 131-139.
- Edwards, F.W. 1932. Diptera. Fam. Culicidae. In: P. Wytsman (ed.), *Genera Insectorum*. Desmet-Verteneuil, Brussels. Fasc. 194. 258 pp, 5 pl.
- Hii, J.L.K. 1985. Genetic investigations of laboratory stocks of the complex of *Anopheles balabacensis* Baisas (Diptera: Culicidae). *Bull. Entomol. Res.* 75: 185-197.
- Hii, J.L.K., E.L. Peyton and V.Y. Shang. 1988. Redescription of the adult and first descriptions of the larva and pupa of *Anopheles (Cellia) sulawesi* Waktoedi, a species of the Leucosphyrus Group from Sulawesi, Indonesia (Diptera: Culicidae). *Mosq. Syst.* 20: 41-54.
- International Code of Zoological Nomenclature*. 1985. Third Edition. International Trust for Zoological Nomenclature, London. xx + 338 pp.
- Kanda, T., K. Takai, G.L. Chiang, W.H. Cheong and S. Sucharit. 1981. Hybridization and some biological facts of seven strains of the *Anopheles leucosphyrus* group (Reid 1968). *Jpn. J. Sanit. Zool.* 32: 321-329.
- Knight, K.L. and E.N. Marks. 1952. An annotated checklist of the mosquitoes of the subgenus *Finlaya*, genus *Aedes*. *Proc. U.S. Nat. Mus.* 101: 513-574.
- Kuitert, L.C. and J.D. Hitchcock. 1948. Observations on *Anopheles leucosphyrus* Don. at Shingbwiyang, Burma (Diptera: Culicidae). *Proc. Entomol. Soc. Wash.* 50: 77-82.

- Macan, T.T. 1948. Mosquitoes and malaria in the Kabaw and Kale Valleys, Burma. Bull. Entomol. Res. 39: 237-268.
- McArthur, J. 1947. The transmission of malaria in Borneo. Trans. R. Soc. Trop. Med. Hyg. 40: 537-558.
- McArthur, J. 1951. The importance of *Anopheles leucosphyrus*. Trans. R. Soc. Trop. Med. Hyg. 44: 683-694.
- Mendis, K.M., R.L. Ihalamulla, E.L. Peyton and S. Nanayakkara. 1983(1984). Biology and descriptions of the larva and pupa of *Anopheles (Cellia) elegans* James (1903). Mosq. Syst. 15: 318-324.
- Peyton, E.L. and B.A. Harrison. 1979. *Anopheles (Cellia) dirus*, a new species of the Leucosphyrus Group from Thailand (Diptera: Culicidae). Mosq. Syst. 11: 40-52.
- Peyton, E.L. and B.A. Harrison. 1980. *Anopheles (Cellia) takasagoensis* Morishita 1946, an additional species in the Balabacensis Complex of Southeast Asia (Diptera: Culicidae). Mosq. Syst. 12: 335-347.
- Peyton, E.L. and S. Ramalingam. 1988. *Anopheles (Cellia) nemophilous*, a new species of the Leucosphyrus Group from Peninsular Malaysia and Thailand (Diptera: Culicidae). Mosq. Syst. 20: 272-299.
- Reid, J.A. 1949. A preliminary account of the forms of *Anopheles leucosphyrus* Doenitz (Diptera: Culicidae). Proc. R. Entomol. Soc. Lond. Series B 18: 42-53.
- Reid, J.A. 1968. Anopheline mosquitoes of Malaya and Borneo. Stud. Inst. Med. Res. Malaya 31: 1-502.
- Reid, J.A. and K.L. Knight. 1961. Classification within the subgenus *Anopheles* (Diptera: Culicidae). Ann. Trop. Med. Parasitol. 55: 474-488.
- Rosenberg, R. and N.P. Maheswary. 1982. Forest malaria in Bangladesh. II. Transmission by *Anopheles dirus*. Am. J. Trop. Med. Hyg. 31: 183-191.
- Scanlon, J.E. and U. Sandhinand. 1965. The distribution and biology of *Anopheles balabacensis* in Thailand (Diptera: Culicidae). J. Med. Entomol. 2: 61-69.

Table 1. Subgroups and Species Complexes in the Leucosphyrus Group (nonhierarchical).

LEUCOSPHYRUS GROUP

LEUCOSPHYRUS SUBGROUP, new subgroup

1. *baisasi* Colless, 1957
2. Con Son Form, new form

Leucosphyrus Species Complex, new species complex

3. *balabacensis* Baisas, 1936
4. *introlatus* Colless, 1957
5. *leucosphyrus* A, Baimai et al. 1988b
6. *leucosphyrus* Doenitz, 1901 (=B)

Dirus Species Complex, Peyton and Ramalingam 1988

7. *dirus* Peyton and Harrison, 1979 (=A)
8. *dirus* B, Hii 1982 (unpublished thesis)
9. *dirus* C, Baimai et al. 1984
10. *dirus* D, Baimai et al. 1984
11. *dirus* E, Peyton and Baimai (unpublished)
12. *nemophilous* Peyton and Ramalingam, 1988
13. *takasagoensis* Morishita, 1946

ELEGANS SUBGROUP, new subgroup

1. *elegans* (James), 1903
2. *hackeri* Edwards, 1921
3. *pujutensis* Colless, 1948
4. *sulawesi* Waktoedi, 1954
5. Sumatra species, new species

RIPARIS SUBGROUP, new subgroup

1. *cristatus* King and Baisas, 1936
2. *macarthuri* Colless, 1956
3. *riparis* King and Baisas, 1936
4. Negros Form, Colless, 1956

Fig. 1

